208377US0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

TAKAO IMAEDA ET AL : ATTN: APPLICATION BRANCH

SERIAL NO. NEW U.S. APPLICATION :

FILED: HEREWITH :

FOR: METHOD FOR PRODUCING
ANTIMICROBIAL PROTEIN
AND FUSION PROTEIN

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please amend the specification as shown in the marked-up copy to read as follows:

Page 9, line 20 through page 10, line 3, replace the text in its entirety with the following:

--The type of the antimicrobial protein A is not limited, as long as the antimicrobially active type has a predetermined mode of anintramolecular disulfide bond and it is a basic antimicrobial protein. Preferable examples include any one of thionin, PR protein, lipid transfer protein, and ribosome-inactivating protein, all from plants, or any one of difensin from plants, insects and humans. The thionin from plants is preferably for example thionin from barley, and more specifically thionin

comprising amino acids at positions 10 to 54 in the amino acid sequence shown as SEQ ID No: 2.--

Page 10, line 21 through page 11, line 2 replace the text in its entirety with the following:

--Preferred examples of the PDI include PDI derived from Fumicola insolens, specifically PDI comprising amino acids at positions 59 to 543 in the amino acid sequence shown as SEQ ID No: 5. Preferred examples of the acid protein include an acid protein encoded by DNA downstream of the nucleotide sequence of thionin derived from barley, specifically an acid protein comprising amino acids at positions 61 to 124 in the amino acid sequence shown as SEQ ID No: 2.--

Page 12, lines 2-17, replace the text in its entirety with the following:

--The coding embodiments include the following. The DNA may encode the antimicrobial protein A and the partner protein B, successively, as a single structural gene. The DNA may encode the antimicrobial protein A, the acid partner protein B1 and the chaperon partner protein B2, successively, as a single structural gene. The DNA may encode the antimicrobial protein A, the intermediate oligopeptide moiety cleavable with appropriate means and the partner protein B, successively, as a single structural gene. The DNA may encode the antimicrobial protein A, the intermediate oligopeptide moiety cleavable with appropriate means, the acid partner protein B1 and the chaperon partner protein B2, successively, as a single structural gene. The antimicrobial protein A and the partner protein B may be encoded as different structural genes. Two or more of the antimicrobial protein A, the acid partner protein B1 and the chaperon partner protein B2 may be encoded as different

structural genes. Such DNA includes for example DNA of the nucleotide sequence shown as SEQ ID No: 1 or 4.--

Page 15, line 17 through page 16, line 1, replace the text in its entirety with the following:

--The thionin gene from barley is of the nucleotide sequence at position 25 to position 159 in SEQ ID No: 4. The PDI gene is of the nucleotide sequence at position 175 to position 1629 in SEQ ID No: 4. In SEQ ID No: 4, the nucleotide sequence CATATG at position 7 to position 12 corresponds to a cleavage region with restriction endonuclease NdeI, while the nucleotide sequence GGATCC at position 1635 to 1640 corresponds to a cleavage region with restriction endonuclease BamHI. In the amino acid sequence concurrently depicted (SEQ ID NO:5), the amino acid sequence Thr-Glu-Gly-Arg at position 5 to 8 and position 55 to 58 corresponds to a recognition site of Factor Xa.--

Page 16, lines 4-14, replace the text in its entirety with the following:

--The gene encoding thionin derived from barley was inserted in a site scissored out via the cleavage with restriction endonucleases NdeI and BamHI in the plasmid pET-19b manufactured by Novagen for cloning. The thionin gene from barley is of the nucleotide sequence at position 28 to position 162 in SEQ ID No: 7. In SEQ ID No: 7, the nucleotide sequence CATATG at position 7 to position 12 corresponds to a cleavage region with restriction endonuclease NdeI, while the nucleotide sequence GGATCC at position 168 to 173 corresponds to a cleavage region with restriction endonuclease BamHI. In the amino acid sequence concurrently depicted (SEQ ID NO:8), alternatively, the amino acid sequence Thr-Glu-Gly-Arg at position 5 to 8 corresponds to a recognition site of Factor Xa.--

Page 17, lines 1-10, replace the text in its entirety with the following:

--The thionin gene from barley is of the nucleotide sequence at position 25 to position 159 in SEQ ID No: 10. The gene of the acid region is of the nucleotide sequence at position 175 to position 264. In SEQ ID No: 10, the nucleotide sequence CATATG at position 7 to position 12 corresponds to a cleavage region with restriction endonuclease NdeI, while the nucleotide sequence GGATCC at position 270 to 275 corresponds to a cleavage region with restriction endonuclease BamHI. In the amino acid sequence concurrently depicted (SEQ ID NO:11), the amino acid sequence Thr-Glu-Gly-Arg at position 5 to 8 and position 35 to 38 corresponds to a recognition site of Factor Xa.--

Page 19, lines 4-9, replace the text in its entirety with the following:

--As shown in SEQ ID No: 1, 4 or 10, the fusion proteins of Examples 1 and 2 and Comparative Example 2 thus dialyzed retained the recognition sequence of a protease Factor Xa in between thionin and the partner protein. Then, the fusion protein was subjected to cleavage with Factor Xa of 30 μg per 1 mg of each fusion protein described above at 30 °C overnight.--

REMARKS

A paper copy of the Sequence Listing is attached to the specification as filed. The specification has been amended to correspond the sequence identifiers (SEQ ID NO:) with the sequences in the Sequence Listing. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the Sequence Listing as attached to the specification. Applicants submit that the application is now ready for examination on the merits

Respectfully submitted,

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Serial No.: New U.S. Application
Amendment Filed: May 25, 01

IN THE SPECIFICATION

Please amend the specification as follows:

Page 9, line 20 through page 10, line 3, replace the text in its entirety with the following:

--The type of the antimicrobial protein A is not limited, as long as the antimicrobially active type has a predetermined mode of anintramolecular disulfide bond and it is a basic antimicrobial protein. Preferable examples include any one of thionin, PR protein, lipid transfer protein, and ribosome-inactivating protein, all from plants, or any one of difensin from plants, insects and humans. The thionin from plants is preferably for example thionin from barley, and more specifically thionin comprising amino acids at positions 10 to 54 in the amino acid sequence shown as SEQ ID No: [1]2.--

Page 10, line 21 through page 11, line 2 replace the text in its entirety with the following:

--Preferred examples of the PDI include PDI derived from Fumicola insolens, specifically PDI comprising amino acids at positions 59 to 543 in the amino acid sequence shown as SEQ ID No: [2]5. Preferred examples of the acid protein include an acid protein encoded by DNA downstream of the nucleotide sequence of thionin derived from barley, specifically an acid protein comprising amino acids at positions 61 to 124 in the amino acid sequence shown as SEQ ID No: [1]2.--

Page 12, lines 2-17, replace the text in its entirety with the following:

--The coding embodiments include the following. The DNA may encode the antimicrobial protein A and the partner protein B, successively, as a single structural gene. The DNA may encode the antimicrobial protein A, the acid partner protein B1 and the chaperon partner protein B2, successively, as a single structural gene. The DNA may encode the antimicrobial protein A, the intermediate oligopeptide moiety cleavable with appropriate means and the partner protein B, successively, as a single structural gene. The DNA may encode the antimicrobial protein A, the intermediate oligopeptide moiety cleavable with appropriate means, the acid partner protein B1 and the chaperon partner protein B2, successively, as a single structural gene. The antimicrobial protein A and the partner protein B may be encoded as different structural genes. Two or more of the antimicrobial protein A, the acid partner protein B1 and the chaperon partner protein B2 may be encoded as different structural genes. Such DNA includes for example DNA of the nucleotide sequence shown as SEQ ID No: 1 or [2] 4.--

Page 15, line 17 through page 16, line 1, replace the text in its entirety with the following:

--The thionin gene from barley is of the nucleotide sequence at position 25 to position 159 in SEQ ID No: [2]4. The PDI gene is of the nucleotide sequence at position 175 to position 1629 in SEQ ID No: [2]4. In SEQ ID No: [2]4, the nucleotide sequence CATATG at position 7 to position 12 corresponds to a cleavage region with restriction endonuclease NdeI, while the nucleotide sequence GGATCC at position 1635 to 1640 corresponds to a cleavage region with restriction endonuclease BamHI. In the amino acid sequence concurrently depicted (SEQ ID

NO:5), the amino acid sequence Thr-Glu-Gly-Arg at position 5 to 8 and position 55 to 58 corresponds to a recognition site of Factor Xa.--

Page 16, lines 4-14, replace the text in its entirety with the following:

--The gene encoding thionin derived from barley was inserted in a site scissored out via the cleavage with restriction endonucleases NdeI and BamHI in the plasmid pET-19b manufactured by Novagen for cloning. The thionin gene from barley is of the nucleotide sequence at position 28 to position 162 in SEQ ID No: [3] 7. In SEQ ID No: [3] 7, the nucleotide sequence CATATG at position 7 to position 12 corresponds to a cleavage region with restriction endonuclease NdeI, while the nucleotide sequence GGATCC at position 168 to 173 corresponds to a cleavage region with restriction endonuclease BamHI. In the amino acid sequence concurrently depicted (SEQ ID NO:8), alternatively, the amino acid sequence Thr-Glu-Gly-Arg at position 5 to 8 corresponds to a recognition site of Factor Xa.--

Page 17, lines 1-10, replace the text in its entirety with the following:

--The thionin gene from barley is of the nucleotide sequence at position 25 to position 159 in SEQ ID No: [4] 10. The gene of the acid region is of the nucleotide sequence at position 175 to position 264. In SEQ ID No: [4] 10, the nucleotide sequence CATATG at position 7 to position 12 corresponds to a cleavage region with restriction endonuclease NdeI, while the nucleotide sequence GGATCC at position 270 to 275 corresponds to a cleavage region with restriction endonuclease BamHI. In the amino acid sequence concurrently depicted (SEQ ID NO:11), the amino acid sequence Thr-Glu-Gly-Arg at position 5 to 8 and position 35 to 38 corresponds to a recognition site of Factor Xa.--

Page 19, lines 4-9, replace the text in its entirety with the following:

--As shown in SEQ ID No: 1, [2 or 4] 4 or 10, the fusion proteins of Examples 1 and 2 and Comparative Example 2 thus dialyzed retained the recognition sequence of a protease Factor Xa in between thionin and the partner protein. Then, the fusion protein was subjected to cleavage with Factor Xa of 30 μg per 1 mg of each fusion protein described above at 30 °C overnight.--